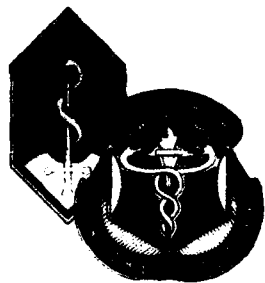


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*Institute Report No. 302*

**Mutagenic Potential of Permethrin  
in the *Drosophila melanogaster*  
Sex-Linked Recessive Lethal Test**

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**GENETIC TOXICOLOGY BRANCH  
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# ABSTRACT

Permethrin, an insecticide/repellent being considered by the military for impregnating the military Battle Dress Uniform (BDU) and for packaging Meal-Ready-to-Eat (MRE), was evaluated for mutagenic potential in the *Drosophila melanogaster* Sex-Linked Recessive Lethal test. Permethrin was determined to be nonmutagenic under conditions of this study.

Key Words: Mutagenicity, Toxicology, Sex-Linked Recessive Lethal Assay, *Drosophila melanogaster*, Permethrin

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## PREFACE

TYPE REPORT: *Drosophila melanogaster* Sex-Linked Recessive  
Lethal Assay

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Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command  
Letterman Army Institute of Research  
Presidio of San Francisco, CA 94129-6800

PROJECT/WORK UNIT/APC: Development of Repellents Against  
Medically Important  
Arthropods/201/WU081

GLP STUDY NUMBER: 84029

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: CPT Zia A. Mehr, MS, MSC

CO-PRINCIPAL INVESTIGATORS: SP4 Paul D. Mauk, BS  
SSG James D. Justus, MS  
MAJ Raj K. Gupta, PhD, MSC

REPORT AND DATA MANAGEMENT: A copy of the final report, study  
protocol, test compound sample,  
raw data, and SOPs will be  
retained in the LAIR Archives.

TEST SUBSTANCE: Permethrin

INCLUSIVE STUDY DATES: 31 July 1984 - 21 January 1985


OBJECTIVE: The objective of this study was to evaluate  
the mutagenic potential of Permethrin using  
the *Drosophila melanogaster* Sex-Linked  
Recessive Lethal Assay.


#### ACKNOWLEDGMENT

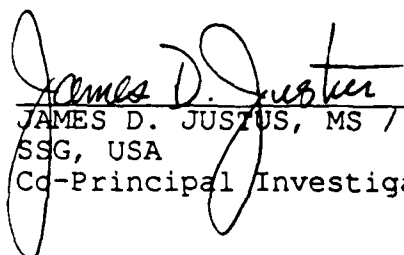
This report is dedicated in memory of SP4 Paul D. Mauk, BS. The investigators wish to thank Conrad R. Wheeler, PhD, for assistance with the formulation of the test compound and to Ms. Mara W. Joshua for secretarial and typing assistance.

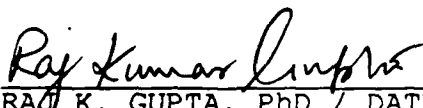
**Signatures of Principal Scientists  
Involved in the Study**

We, the undersigned, declare that the GLP Study 84029 was performed under our supervision, according to the procedures described herein, and that the report is an accurate record of the results obtained.

 27 July 88  
DON W. KORTE JR., PhD / DATE  
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Study Director

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27 July 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84029

1. This is to certify that in relation to LAIR GLP Study 84029, the following inspections were made:

31 July 1984	- Dosing
05 August 1984	- Dosing
07 September 1984	- Brood 4 Mating, 2nd Run

2. The raw data and the institute report entitled "Mutagenic Potential of Permethrin in the *Drosophila melanogaster* Sex-linked Recessive Lethal Test, Toxicology Series 102, were audited on 28 June 1988.

*Walter G. Bell*

WALTER G. BELL

SFC, USA

Quality Assurance Auditor

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**Mutagenic Potential of Permethrin in the  
*Drosophila melanogaster* Sex-Linked Recessive  
Lethal Test -- Mehr et al.**

**INTRODUCTION**

Permethrin is an insecticide/repellent being considered by the military for impregnating the military Battle Dress Uniform (BDU) and for packaging Meal-Ready-to-Eat (MRE). These proposed uses would subject many soldiers to long-term exposure to minute quantities of Permethrin. Recently, it has been suggested that Permethrin may be a mutagen since a borderline positive response in a mouse bone marrow assay has been reported (1). This study evaluated mutagenic potential of Permethrin in the *Drosophila melanogaster* Sex-Linked Recessive Lethal (SLRL) Assay.

Rationale for SLRL Testing

A variety of tests using *Drosophila melanogaster* are available for the detection of specific types of genetic changes. The most sensitive assay that detects the broadest range of mutations is the SLRL test (2-4). This test has also been called the Basc or Muller-5 test (5, 6). This test uses insects of a known genotype and detects lethal mutagenic changes in 800 to 1000 loci on the X-chromosome, representing 80% of the X-chromosome or 20% of the entire genome (5,7). The SLRL test is used frequently to assess the mutagenic response of *Drosophila melanogaster* to test substances (2,4,7).

### Genetic Basis of the SLRL Test

The basic mechanism of the SLRL test is that the X-chromosome of the father is passed on to the daughter; the sons receive their X-chromosome from the mother. Therefore, the recessive lethal mutations located on the X-chromosomes are expressed in males in a hemizygous condition, i.e. the Y-chromosome does not contain the dominant, wild-type alleles to suppress the manifestation of recessive lethal mutations.

Consequently, among the progeny of a female carrying a recessive lethal mutation on one of her X-chromosomes (heterozygous for a recessive lethal mutation), half of the male progeny die. By using suitable genetic markers, the class of males carrying the mutated X-chromosomes of treated grandfathers can be determined easily. If a lethal mutation were induced, this class would be missing and its absence easily scored. The assay system must use strains in which crossing-over in females is prevented since transfer of the lethal mutation from the paternal to the maternal X-chromosome by genetic recombination would suppress its expression. The crossing-over would lead to erroneous study results because males receiving that X-chromosome would survive. Since combinations of suitable inversions effectively inhibit crossing-over, females used for the SLRL test carry two scute inversions: the left-hand part of scS1 and the right-hand part of sc8 covering the whole X-chromosome and a smaller inversion In-S in the Basc chromosome (5).

### Description of Test

The SLRL test (8) was developed in 1948 for determining genetic changes that kill the developing individual (egg to pre-adult stage) in the hemizygous, but not homozygous or heterozygous, conditions. Such genetic changes, i.e. recessive lethal mutations, can be induced on all chromosomes. Only two test generations are needed to detect whether sex-linked recessive lethal mutations have been induced on the X-chromosome.

In the test, males with normal round red eyes (Canton-S (CS)) whose chromosomes contain wild-type alleles are exposed to Permethrin. Such an exposure will produce a recessive lethal mutation if the X-chromosome is affected. These males are mated to homozygous Basc females. The Basc phenotype is characterized by bar (narrow-shaped) eyes which are

apricot in color. The bar eyes serve as a genetic marker for the homozygous and hemizygous genotypes since in the heterozygous expression the eyes are kidney-shaped. The progeny of this cross (CS males X Basc females) consists of females heterozygous for the treated X-chromosome, characterized by kidney-shaped red eyes, and males of the Basc phenotype that have received their X-chromosome from their Basc mother. Each F1 female possesses one paternal X-chromosome which was exposed to the test compound in the male gamete. F1 siblings are allowed to mate; they produce the F2 generation. The F2 generation will consist of males of two phenotypic expressions and females of two phenotypic expressions. The male phenotypes have round red eyes (hemizygous carrying the treated X-chromosome from the F1 female) or bar-shaped apricot eyes (hemizygous for the Basc chromosome). The female phenotypes carry the chromosomes for red eyes (heterozygous, carrying the treated X-chromosomes from the F1 female and the Basc chromosome) or chromosomes for bar-shaped apricot eyes (homozygous for the Basc chromosome). The F2 generation is then inspected for the presence of males with round eyes. If this class is missing, it can be concluded that the treated male gamete contained a recessive lethal mutation. Thus, this test relies upon the disappearance of a whole Mendelian class (males with round red eyes).

A brooding technique was used to sample sperm cells exposed to the test chemical during different stages of spermatogenesis because chemicals often exhibit stage specific mutagenicity. Brooding was accomplished by transferring the treated males to vials containing fresh virgin females at intervals of 1, 4, 6, and 8 days after completion of the dosing period. This technique assures that the four broods of females are inseminated with sperm exposed to the test chemical during successive stages of germ cell development: Brood 1 = mature sperm (Days 1-3); Brood 2 = primarily spermatids (Days 4-5); Brood 3 = primarily meiotic stages (Days 6-7); and Brood 4 = primarily spermatogonia (Days 8-10). This procedure safeguards against the possibility that chemicals with more pronounced effects in earlier stages of spermatogenesis are not dismissed erroneously as false negatives.

### Objective of Study

The objective of this study was to evaluate the mutagenic potential of Permethrin in the *Drosophila melanogaster* Sex-Linked Recessive Lethal assay.

## **MATERIALS AND METHODS**

### Test Substance

Chemical Name: Permethrin

LAIR Code No. TP031

Molecular formula:  $C_{21}H_{20}Cl_2O_3$

Other test substance information is presented in Appendix A.

### Vehicle

A suitable vehicle was found by testing the solubility, stability, and toxicity of a number of compounds. A mixture of 0.4% Tween 80, 0.2% ethanol, and 1.0% fructose in water was found to be an appropriate vehicle for the Permethrin as it had no appreciable effect on the test insects. Use of dimethyl sulfoxide (DMSO) as a solvent was avoided as recommended by the EPA (9).

### Test Model

Insect Genus and Species: *Drosophila melanogaster*

Strains: Canton-S (CS), a wild-type stock, characterized by round red eyes, was selected for mutagenicity studies because it has shown a low spontaneous mutation frequency (10).

Basc, a laboratory stock, homozygous in females, possesses bar-shaped, apricot-colored eyes and scute as phenotypic markers. The genetic designation is  $In(1)sc^{Sl}Lsc^{8R}IN(1)S, sc^8, sc^{Sl}waB$ .

Both strains are presently being reared in the insectary at Letterman Army Institute of Research. The original stock colonies were obtained from the Mid-American *Drosophila* Stock Center, Bowling Green State University, Bowling Green, Ohio.

#### Diet

The diet was the standard medium consisting of cornmeal (NBCO Chemicals), unsulphured molasses (Ingredient Technology Corp.), yeast (Nabisco Brands, Inc.), and nutrient agar (Moorhead & Co., Inc.) used for colony rearing of *Drosophila melanogaster*. A materials list and instructions for its preparation are contained in LAIR SOP-OP-STX-5 "Drosophila Media Preparation."

#### Restraint

Ether (J. T. Baker Chemical Co.) anesthesia was used to restrain flies being collected for mating and for general colony maintenance.

#### Identification System

Each CS male from the 72-hour LC<sub>50</sub> exposure (test, negative, and positive control) was assigned a unique number. This number was also placed on the vial in which its progeny was produced (LAIR SOP-OP-STX-8 "Sex-Linked Recessive Lethal (SLRL) *Drosophila melanogaster* Mutagenicity Test"). In this manner progeny could be traced back to the parental male which had been subjected to the test compound or control vehicle.

#### Storage of Raw Data

Tabular data from this study (GLP 84029) for each male are in the archives of Letterman Army Institute of Research, Presidio of San Francisco, California.

#### Environmental Conditions

All studies were conducted within the insectary at a temperature of  $23 \pm 1^{\circ}\text{C}$ , relative humidity of  $48 \pm 8\%$  and a photoperiod of 12 hours light and 12 hours dark. All insect colonies were reared in polypropylene bottles, and those used in the SLRL testing were housed in glass vials (LAIR SOP-OP-STX-6 "Drosophila Stock Colony Maintenance").

#### Dosing

A number of preliminary studies were conducted to test potential vehicles for toxicity to flies and for their ability to solubilize and stabilize Permethrin.

To determine the stability of Permethrin in the 1 mM stock solution, a 3-day-old solution was analyzed for one of the products of hydrolysis, 3-phenoxybenzyl alcohol. No trace of 3-phenoxybenzyl alcohol could be detected by gas chromatography, thus demonstrating that the compound was stable under these conditions. The pH of both the stock solution and negative control throughout the assay ranged from 5.8 to 6.5.

Dosing was accomplished in compliance with LAIR SOP OP-STX-7 "*Drosophila melanogaster* Exposure Procedures." The test compound solutions were prepared daily just before dosing. The CS strain (wild-type) males were allowed to feed on 250  $\mu$ l of various concentrations of the Permethrin formulated with 0.4% Tween 80, 0.2% ethanol, and 1.0% fructose in water. Dosing was continuous for 72 hours. Flies were transferred every 24 hours to vials containing freshly prepared dosing media. The concentrations used for the LC50 determinations were 10, 4, 3, 2, and 1  $\mu$ M Permethrin. The LC50 determination was conducted 4 times, once for each replicate. The concentration for each replicate that produced a 72-hour mortality closest to 50% was designated as the approximate LC50 (7), and only flies raised on this concentration were used in subsequent crosses. Concurrent exposure to 0.4% Tween 80, 0.2% ethanol, and 1.0% fructose in water was designated as the negative control group. A positive control group was exposed to a 1.0 mM ethylmethane sulfonate solution formulated with 1.0% fructose in water. Ethylmethane sulfonate is a known mutagen and was used to confirm the ability of the test organism to produce SLRL mutations (11).

#### Test Format

The CS males surviving the LC50 (approximate) dose of the test chemical and those males subjected to the concurrent negative controls were used in the SLRL assay. Twenty-five CS male (wild-type) survivors of the test chemical and negative control compound were scored by mating to Basc virgin females (Basc chromosomes). This procedure was accomplished by placing 3 Basc virgin females in a vial with one CS male. The vial was labeled with the male's unique number. At days 1, 4, 6, and 8 after dosing the CS male was transferred to successive groups of 3 Basc virgin females in vials with that male's unique number. These intervals corresponded to broods 1, 2, 3, and 4. This procedure was replicated 4 times. Scoring of the mutants resulting from positive

control exposure was based on mating 5 CS males in the same manner as males treated with the test compound. This procedure was also replicated 4 times. After sufficient numbers of flies had emerged, a maximum of 25 (minimum of 5) kidney-shaped red-eyed F1 females were selected at random and mated with their sibling white-body, bar-shaped, apricot-eyed males. Each pair was placed in an individual vial, and these vials from the same uniquely numbered father were placed together and labeled with his unique number for reference. After 2 to 3 weeks the F2 progeny were examined and scored for the absence of round, red-eyed males, which would indicate that a lethal mutation had taken place in the treated male. Confirmation of a lethal mutation was obtained by conducting an F3 cross from each vial scored as a lethal mutation. This was accomplished by crossing three F2 females (kidney-shaped red eyes) with three males with bar-shaped white-apricot eyes, in three separate vials (one male and one female per vial). Absence of males with round, red eyes in the resulting F3 generation confirmed the existence of a recessive lethal mutation. Experimental conclusions were based on the spontaneous mutation frequency (negative control) compared to the mutation frequency induced by the test chemical. This entire procedure was replicated 4 times to obtain a sufficiently large sample.

#### Historical Listing of Significant Study Events

Appendix B is a historical listing of significant study events.

#### Statistical Analysis

This testing was designed to examine approximately 2500 X-chromosomes in each of 4 replications, thereby yielding a total of 8000 to 10,000 X-chromosomes for examination. Vials without F2 progeny or fewer than 5 progeny (F2) were scored as failures. The BMDP (Biomedical Programs) computer package was used to perform the analyses (11). Based on the number of lethal and nonlethal offspring for each male, by combining all replicates, the mutation frequency of Permethrin was compared to that of the control by Fisher's exact test for each of the four broods separately and for the combined broods. All statistical tests were conducted at the 0.05 level of significance.

Deviations from SOP/Protocol

No deviations from SOPs and/or GLP Protocol 84029 were made during this study.

Use of dimethyl sulfoxide (DMSO) as a solvent was avoided as recommended by the EPA (13).

**RESULTS**

The percent mortalities corresponding to the concentrations of Permethrin that most closely approximated the LC50 after a 72-hour exposure are shown for each replication in Table 1. These concentrations were selected for use in the appropriate replication because they gave the closest approximation to the LC50 of the concentrations used in each pilot study.

The frequencies of spontaneous mutation for Permethrin and the negative control were 0.135% and 0.133% based on 8874 and 9001 X-chromosomes, respectively. The mutation frequency for the positive control, 1-mM ethylmethane sulfonate, was 12.6%. The mutation frequencies for each compound are presented in Table 2. No significant difference was detected between the mutation frequency of the negative control and the Permethrin with the Fisher's exact test ( $p = 1.00$ ). Also, no significant differences were detected between the negative control and the Permethrin for the data of broods 1, 2, 3, and 4 (Table 3).



TABLE 1

Concentration of Permethrin Fed to CS Males and  
Corresponding Mean Percentage Mortality for Each  
Replication in the Sex-Linked Recessive Lethal  
Assay

Replication Number	Concentration $\mu\text{M}$	%Mortality* $\pm$ s.d.
1	2.0	58.0 $\pm$ 25.7
2	3.0	63.0 $\pm$ 27.5
3	2.0	41.4 $\pm$ 18.6
4	3.0	60.0 $\pm$ 30.2

\* Based on sample size of 100 CS males.

Table 2

PERCENT MUTATION FREQUENCIES IN THE SEX-LINKED  
RECESSIVE LETHAL ASSAY OF PERMETHRIN\*

Compound	1	2	3	4	Total Mutations	(Percent) Mutations
Permethrin	2/1923	2/2330	4/2205	4/2416	12/8874	(0.135)
Negative Control	4/2086	3/2396	3/2245	2/2274	12/9001	(0.133)
Positive Control	29/230	35/372	12/94	51/311	127/1007	(12.60)

\*Data are recorded as number of SLRL events/number of X-chromosomes tested.

Permethrin: 25 male *Drosophila melanogaster* flies (CS strain) formed the P generation.

Negative Control: 25 male *Drosophila melanogaster* flies (CS Strain) formed the P generation.

Positive Control: 5 male *Drosophila melanogaster* flies formed the P generation.

Table 3

FISHER'S EXACT TEST FOR SIGNIFICANCE OF THE  
DIFFERENCE BETWEEN PERMETHRIN AND NEGATIVE  
CONTROL IN SEX-LINKED RECESSIVE LETHAL ASSAY

Compound	Brood Number			
	1	2	3	4
Permethrin	6/2228	3/2247	3/2223	0/2176
Negative Control	4/2343	4/2326	1/2192	3/2140
Positive Controls	54/289	41/250	24/204	8/264
p values	0.5394	1.0000	0.6249	0.1221

Permethrin: Permethrin was formulated with 0.4% Tween 80, 0.2% ethanol, and 1% fructose solution in deionized H<sub>2</sub>O. Data are from 25 male *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc strain female flies each.

Negative Control: 0.4% Tween 80, 0.2% ethanol, and 1% fructose in deionized water. Data are from 25 *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

Positive Control: 1.0 mM ethylmethane sulfonate and 0.4% Tween 80, 0.2% ethanol, and 1% fructose in deionized H<sub>2</sub>O. Data are from 5 male *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

## DISCUSSION

These findings are consistent with results of other studies which evaluated the mutagenic potential of Permethrin in various bioassays. Permethrin was reported to be non-mutagenic in the *Salmonella typhimurium* Plate Incorporation and the *Escherichia coli* Reverse Mutation assays for Point/Gene Mutations in prokaryotes and in the *Saccharomyces cerevisiae* D3 and the Human Lung Fibroblast Unscheduled DNA Synthesis assays for Primary DNA Damage in eukaryotes (12). Permethrin was also reported to be nonmutagenic in V79 Chinese hamster cells both in the presence and absence of primary rat hepatocytes (13). However, results from an *in vivo* assay in mouse bone marrow cells suggested that Permethrin was a weak mutagen as it caused aberrations in 5% of the examined mitoses in contrast to 2% in control animals (1). The SLRL assay was chosen to evaluate the mutagenic potential of Permethrin because it is an *in vivo* assay that requires a large sample size which enables expression of a weak mutagen. In addition, an invertebrate model was selected since poikilothermic animals are selectively sensitive to pyrethroid compounds such as Permethrin (1). The absolute lack of a mutagenic effect in this assay coupled with the preponderance of evidence from *in vitro* studies strongly suggests that Permethrin has little potential to cause genetic damage in humans.

## CONCLUSION

The results of this study indicate that Permethrin is not mutagenic when evaluated in the *Drosophila melanogaster* sex-linked recessive lethal assay.

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## APPENDICES

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**Appendix A: CHEMICAL DATA**

Chemical name: 3-(2,2-Dichloroethenyl)-2,2-dimethyl  
cyclo-propanecarboxylic acid (3-phen  
oxyphenyl) methyl ester

Trade name: Technical Grade Permethrin

Chemical Abstracts Service registry no.: 52645-53-1

LAIR Code Number: TP31

Molecular formula:  $C_{21}H_{20}Cl_2O_3$

Molecular weight: 391.3

Names of contaminants and percentages:  $84.8 \pm 2 \%$ \*

Manufacturer/Source: McLaughlin, Gormbey, King Co.  
Minneapolis, MN 55427

Manufacturer's Lot No.: 178-78

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\*Ho, Bert, Military toxicologic testing. Laboratory  
Notebook N. 85-03-009, p. 18-20. Letterman Army  
Institute of Research. Presidio of San Francisco, CA  
94129-6800.



**Appendix A (Contd.): CHEMICAL DATA****Stability of the Permethrin Stock Solution\***

Permethrin is a photostable synthetic pyrethoid; however, it is susceptible to cleavage at the ester linkage under certain conditions leading to the formation of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzyl alcohol (3-PBA). For this reason it was necessary to examine the extent of hydrolysis occurring in the 1 mM stock solution.

A period of three days was chosen as the working life of the solution. The amount of hydrolysis was determined by extracting a stock solution of Permethrin 72 hours after preparation and analyzing the extract for 3-phenoxybenzyl alcohol by gas chromatography. As a control a second solution of Permethrin was prepared and spiked with 1 mg of 3-PBA. This solution was extracted immediately after preparation and analyzed by GC. A comparison of the retention times for the chromatogram peaks of the two extracts to those obtained for pure Permethrin (91.1%) and 3-PBA standards demonstrated that: 1) 1 mg of 3-PBA (representing a 2% hydrolysis) could be extracted and easily detected by GC, and 2) no trace of 3-PBA could be detected in the three-day-old stock solution of Permethrin. Since the actual detection limit for 3-PBA was well below the quantity representing a 2% decomposition, the results demonstrate essentially no decomposition of Permethrin after 72 hours.

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\*Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook N. 84-05-010, p. 9-20. Letterman Army Institute of Research. Presidio of San Francisco, CA 94129-6800.

## Appendix A (Contd.): CHEMICAL DATA

### Synthesis of 3-Phenoxybenzoic Acid Standard

3-Phenoxybenzoic acid was obtained from the hydrolysis of Permethrin using the method of Gaughan et al.\* A solution of Permethrin (1 g) in methanolic sodium hydroxide (1 N, 10 ml) was stirred for 36 hours and partitioned between carbon tetrachloride (3 x 30 ml) and sodium hydroxide (2 N, 50 ml). The organic extracts were pooled, back extracted with sodium hydroxide (2 N, 25 ml), and dried over sodium sulfate. The solution was filtered and the solvent removed *in vacuo*. A sample of the remaining yellow oil was analyzed using a Kratos MS-25 mass spectrometer via direct insertion probe under the following conditions: ionizing voltage, 70 eV; source temperature 225° C; scan time 3 seconds per decade; scan range 0-600 AMU. The intense ion observed at  $m/z$  200 was the correct molecular ion for 3-phenoxybenzoic acid.

### Gas Chromatography Assay

The gas chromatographic (GC) assay was developed on a Varian model 4600 GC with a Varian CDS 401 Vista controller. A 2-m column packed with 20% SP 2340 on 100/120 Chromosorb W AW, lot #F0774 from Supelco was employed under the following conditions: carrier gas, nitrogen, 20 ml/min; column temperature 240°C; injector and detector temperature 275° C; attenuation 16. Reproducible retention times of 5.7 min for 3-phenoxybenzyl alcohol and 14.5 and 17 min for the isomers of Permethrin were obtained.

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\*Gaughan LC, Unai T, and Casida JE. Permethrin metabolism in rats. J Agric Food Chem 25 (1):p-17 (1977).

**Appendix A (Contd.): CHEMICAL DATA****Preparation and Extraction of Permethrin and  
Permethrin/3-Phenoxybenzoic Acid Solutions**

A stock solution of Permethrin (250 ml, 1 mM) was prepared as described on day one and again on day four of the study. Two stock solutions of Permethrin (250 ml) were prepared as described, one on day one and one on day four of the study. The first solution was allowed to sit for 72 hours before being extracted with heptane (3 x 50 ml). Centrifugation was required for each extraction to resolve the emulsion. The heptane extracts were pooled and washed with a dilute solution of sodium chloride (5%, 2 x 100 ml) followed by a saturated solution of sodium chloride (1 x 100 ml). The organic extract was dried over sodium sulfate and the solvent was removed in vacuo. Acetone (1 ml) was added and the solution was transferred to a screw-capped test tube with a Teflon liner. The solution was stored at -5 C° prior to analysis by GC on the fifth day of the study.

After preparation of the stock solution on day four of the study, 3-phenoxybenzyl alcohol (1 mg in 0.2 ml acetone) was added, and the solution was extracted immediately as described above. The final solution in acetone was similarly stored and analyzed the following day.

**GC Analysis of Stock Solution Extracts**

One microliter of the stock solution extracts and a standard mixture (3-phenoxybenzyl alcohol, 1 mg/ml and Permethrin, 5 mg/ml in acetone) were analyzed by GC. A peak at 4.7 min was readily observed in the chromatogram of the spiked sample; however, no peak corresponding to 3-phenoxybenzyl alcohol was present in the extract of the 72-hour solution of Permethrin. Peaks corresponding to the isomers of Permethrin were observed for each extract.

**Appendix B. HISTORICAL LISTING OF SIGNIFICANT EVENTS**

<u>Date</u>	<u>Event</u>
31 July - 3 Aug 84	Begin Replicate 1 (Run 53).
28 Aug - 31 Aug 84	Begin Replicate 2 (Run 54).
30 Oct - 2 Nov 84	Begin Replicate 3 (Run 55).
13 Nov - 16 Nov 84	Begin Replicate 4 (Run 56).

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